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Cytology, morphology and amino acid characterization of a putative Agroelymus and its presumed parents

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CYTOLOGY, MORPHOLOGY AND AMINO ACID
CHARACTERIZATION OF A PUTATIVE <u>AGROELYMUS</u>
AND ITS PRESUMED PARENTS

by

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INTRODUCTION

In the summer of 1949 a number of grass plants were observed in the vicinity of Palmer, Alaska (Latitude 61.5° N., Longitude 149.5° W.) that were atypical of any known existing species. Several colonies were found by the author and by Lepage (16). Since that time other colonies have been located and at the present time these plants have been found growing in at least six discontinuous areas within a 15-mile radius of Palmer. Two species, *Elymus canadensis* L. and *Agropyron sericeum* Hitch., always grow in association with these plants and *Agropyron repens* (L.) Beauv. frequently occurs in the same association.

Anderson (3) considered the former species an introduction to Alaska. This conclusion is substantiated by its limited distribution in the Palmer area along avenues of transportation and its relegation to habitats where the natural environment was disturbed and the indigenous flora was destroyed either partially or completely.

Anderson (3) also considers *A. repens* an introduced species although its distribution is much wider than that of *E. canadensis*. The greater variability and competitive ability of *A. repens* has facilitated its spread over most areas in the territory which were settled by man. Because of its aggressive nature and extremely wide adaptability, *A. repens* has been declared a noxious weed in Alaska.

A. sericeum is indigenous to Alaska and is distributed primarily in central Alaska. Along with several other native species it has succeeded in partially revegetating such denuded areas as roadsides and railroad rights-of-way. On these sites its range overlaps that of the exotic *E. canadensis* and plants of intermediate morphological nature occur.

These observations led to the assumption that the intermediate plants are hybrids. Such plants are completely sterile and exhibit apparent heterosis. This assumption led to the present research, the objectives of which were (1) to verify the hybridity of these plants, (2) to determine their parentage and (3) to determine whether the hybrid possesses any economic potential. Cytological, morphological and chemical data which bear on these objectives were collected.

REVIEW OF LITERATURE

Interspecific and intergeneric hybridization are considered commonplace in the Gramineae. Myers (22) reported in 1947 that over 200 instances of such hybridization had been recorded and the number undoubtedly has increased since that date.

According to Jenkin (14) Swayne stated in 1790 that the principal reasons for the great frequency of natural hybrids in the grass family are (1) grasses grow together in dense stands of numerous individuals belonging to various species; (2) they produce large amounts of windborne pollen; and (3) the reproductive organs of different species are much alike. Further, many grasses are spread into new habitats by various vectors often resulting in a few individuals of an exotic species being introduced into the habitat of a previously geographically isolated species. The frequent partial or complete self-incompatability and perennial nature of the exotics together with the exposure to enormous amounts of foreign pollen during the course of their existence are circumstances which greatly favor hybridization.

Interspecific and intergeneric hybridization has been a potent factor in the evolution of many present day species in the Gramineae. Many grasses are considered derivatives from hybridization of other species followed by the spontaneous doubling of the chromosome complement of the hybrid. Within recent years the parentage of many such species has been delineated by Nielsen and Rogler (25), Pohl (26), and Stebbins and others (37), (38), (39).

Many species in the grass family are polyploid and numerous genera have extensive polyploid series. Stebbins (33) recognizes four types of ployploids, namely, autopolyploids, true or genomic allopolyploids, segmental allopolyploids and autoallopolyploids. Autopolyploidy seldom occurs in nature and most polyploids in the *Gramineae* are of the three last mentioned types. These types could arise only through interspecific or intergeneric hybridization.

Many hybrids reported in the grass family involve rather distantly related species. However, when all morphological characteristics are thoroughly analyzed the species involved often do not appear as distant as indicated by our present taxonomic system, which places undue emphasis on certain easily observed characters, particularly those of the inflorescence. Stebbins (35) states that cytogenetic evidence and gross morphology indicate that *Festuca elatior* is much closer to *Lolium perenne* than to other species in its own genus with which it will not hybridize. Similarly in the tribe *Hordeae*, *Agropyron trachycaulum* appears much closer to *Elymus glaucus* than to *A. intermedium*, *A. smithii*,

A. elongatum, or A. cristatum. E. glaucus, A. parishii or A. spicatum appear closer on the basis of cytogenetic evidence than E. glaucus, E. condensatus, E. triticoides, E. mollis or E. erianthus.

Classification of the tribe Hordeae is inherently difficult and many revisions have been proposed. The tribe is characterized by an abundance of interspecific and intergeneric hybridization. As Stebbins has stated (34, p. 252):

> Nearly all plant genera which are intrinsically difficult of classification owe this to either the direct effects of interspecific hybridization or the end results of hybridization accompanied by polyploidy, apomixis or both.

Most interspecific hybrids are quite sterile or have only very low fertility. When even moderate fertility occurs, the most common result of hybridization is the introgression of blocks of genes of one parental species into the genetic system of the other. Backcrossing to one or both parents usually occurs as a result of considerable pollen sterility in the F_1 hybrid and the exposure of its stigmas to an abundance of pollen of the parental species. If an F_2 population does arise, only those individuals with adaptive values similar to the parental species will survive, for gene recombinations that are markedly different are not likely to find a suitable ecologic niche. This in essence is introgressive hybridization, as discussed thoroughly by Anderson (2).

Interspecific hybrids frequently are highly sterile and exhibit irregular meiotic divisions and pollen sterility (30). Successful hybrids often are derived from species with completely non-homologous genomes. Such hybrids have little or no chromosome pairing at meiosis and nearly complete sterility. Such hybrids can produce amphidiploids with high fertility and no interspecific segregation (8). Hybrids with considerable chromosome pairing may produce amphidiploids with lowered fertility (8).

Many interspecific hybrids with apparently normal meiosis exhibit complete sterility. Many such cases were listed by Dobzhansky (11) and Stebbins (34). Sterility of this nature could be either genic or chromosomal but is due in most cases to the existence of structural hybridity for small chromosomal segments. This hypothesis was suggested by Sax (29) in 1933 and has been amplified and designated "cryptic structural hybridity" by Stebbins and co-workers (32) (38). A discussion of this topic is presented by Stebbins (34).

Structural hybridity results from chromosomal inversions and tranlocations that result in chromosomal differences. If these differences are large, multivalent associations will occur at meiosis, but if the segments involved are small, essentially normal pairing occurs. Nevertheless pairing of the dissimilar chromosomes will result in gametes that are inviable because they contain unbalanced, disharmonius combinations of chromosomal material.

Sterility in the F_1 hybrid resulting from structural hybridity can be overcome in the amphidiploid because each chromosome will be present in duplicate and preferential pairing should result in normal, viable gametes. If sterility in the F_1 is genic the amphidiploid would be characterized by the same sterility.

The use of interspecific hybridization as an effective tool for improving forage and range species was advanced by Love (18) and Stebbins (35). Progress has been made in this field but no forage varieties of major economic importance have been produced.

Very few cases are found in the literature in which *Elymus canadensis* is involved in interspecific hybrids. Church (7) reported hybrids between *E. canadensis* and related species of *Elymus* and stated that some may have low fertility. Lepage (16) collected a number of hybrid specimens in Alaska and Canada and on the basis of their external morphology concluded that *E. canadensis* was involved in hybridizations with *Agropyron repens* and *A. sericeum*. He assigned the names X*Agroelymus Hodgsonii* and X*A. palmerensis* respectively to these hybrids.

The generic name X*Agroelymus* was proposed by G. Camus in 1927 (6). The genus was not described and is therefore invalid. Rousseau (28) described and revalidated the generic name in 1952. Lepage (17) amplified the descriptions in 1953 and introduced two new sections, X*Agropsammelymus* and X*Agroclinelymus*. The hybrids X*Agroelymus* *Hodgsonii* and X*A*. *palmerensis* belong to the latter section.

Chromosome numbers of 28 and 42 have been reported for *A*. *repens* and 28 for *E*. *canadensis* by Darlington and Janaki (9). Nielsen and Humphrey (24) found both 28 and 42 chromosome races in *E*. *canadensis*.

Smith (31) reported that *E*. *canadensis* is largely self-pollinated but that a certain amount of cross fertilization occurs in the field. In the same study *A*. *repens* was found to have low self-fertility.

In studies of external morphology of species and suspected interspecific and intergeneric hybrids at least two difficult problems confront the investigator. One is the adequate sampling of the variation that exists in the population and a second is the analysis of the variation. Anderson (1) has proposed the mass collection technique as a means of adequately and randomly sampling the population variation. The same author (2) discussed valuable special techniques such as scatter diagrams, ideographs, hybrid indices, radiate indicators, and photographs for recording and analyzing the variation of the sample.

Davidson (10) recommended the use of polygonal graphs for graphical summarization of characteristics that can be measured quantitatively. This method enables the investigator to depict in one plane the population means of several species for many characteristics, and greatly facilitates comparisons of the various populations.

Specificity for proteins, amino acids, carbohydrates and perhaps other compounds found in plants undoubtedly is one of the important foundations of the differences between species and other groups. Studies on this specificity should help to clarify species differences and, if effective, should prove valuable in determining their phylogenetic relationships.

Mez and Ziegenspeck (20) carried out comprehensive serological tests using plant proteins and developed a phylogenetic tree on that basis. The method involved injecting plant proteins into experimental animals and detecting the production of antibodies by the animal. This work was accepted by some but severely criticized by others. It required considerable expenditure in time and experimental animals and the services of competent biochemists. Therefore this type of work has not been pursued extensively by other workers.

The advent of rapid and accurate chemical procedures, such as chromatography and paper electrophoresis, should give new emphasis to this type of work. Characterization of plant compounds may be extremely valuable as an aid in determining plant relationships.

A beginning already has been made in this direction. Buzzati (5) used chromatographic techniques to demonstrate chemical differences between the heterozygote and both homozygotes for the yellow-green mutant in muskmelon. These differences exhibited a remarkable constancy and a high degree of independence from environmental conditions. The chemical nature of the substances revealed were not determined.

Hunt (13) showed that differences exist between certain legumes with respect to various amino acids at different stages of growth. Barrett and McLaughlin (4) reported that varieties of wheat resistant and susceptible to race 9 of *Puccinia triticina* differ with respect to proteins and amino acids and suggested that susceptibility and resistance might be associated with certain types of plant proteins.

MATERIALS AND METHODS

During the summer of 1950 plants of Agropyron sericeum, Elymus canadensis and the sterile XAgroelymus were obtained from several sites and were established in a nursery. They were space planted in rows, with the hybrid planted in the center row to facilitate possible natural backcrossing.

A natural population of A. repens grew in the immediate proximity of the transplanted material. All plants became established easily and were kept in vigorous condition by liberal fertilization. In each of four growing seasons the hybrid plants were examined to determine whether any seeds were produced.

Six to eight plants of A. sericeum, A. repens, and E. canadensis were grown in pots in the greenhouse. Numerous florets of each were emasculated and pollinated with mature pollen from the other species.

During the summer of 1953 about 15-20 spikes of each species were emasculated using the hot water technique described by Keller (15). Spikes having the lower spikelets ready for anthesis were selected and submerged in water at 46° C. for 1.5, 2.0, and 2.5 minutes. They were bagged immediately and pollinated and rebagged several days later when stigmas were extruded. "Glassine" bags were used in all cases. Untreated spikes also were bagged to serve as checks of seed set under bags, and emasculated but unpollinated spikes were used as checks for effectiveness of pollination. Seeds produced under bag were germinated in moist vermiculite at alternating temperatures of 20 and 30° C. early in the spring of 1954 and the seedlings were transplanted to the field when well established.

Cytological Methods

Spikes selected for cytological examination were fixed in acetic alcohol (3 parts absolute alcohol : 1 part glacial acetic acid) or Carnoy's fixative (6 parts absolute alcohol : 3 parts chloroform : 1 part glacial acetic acid). After fixation they were stored in 70% alcohol at 40° F. Spikes were fixed just prior to emergence of the distal spikelets from the boot. By this time meiosis generally was well under way.

Studies of microsporogenesis were made from aceto-carmine smears. Slides containing desirable figures were made permanent by the Venetian turpentine method described by Wilson (42). Photographs and meiotic data were taken from permanent slides.

Pollen studies were made from mature anthers disected from herbarium specimens in the mass collections. Spikelets of *A. sericeum* and *E. canadensis* were boiled in water to soften tissues. Anthers were disected and then crushed in a drop of lacto-phenol containing a small amount of 1 per cent IKI. Spikelets of the hybrid were softened by the reagent described by Pohl (27). Anthers were disected and then crushed in lacto-phenol containing hemalum to darken the exine and produce sufficient contrast for photography. Photographs of pollen grains were made from temporary slides.

In the spring of 1953 vegetative pieces of five hybrid plants were rooted in vermiculite. When roots were one to two inches long about 1 cm. of the root tips were cut off and the remainder of the roots were immersed for two hours in a 0.2 per cent colchicine solution. After treatment they were washed and returned to vermiculite. The following day the same treatment was repeated, after which the roots were washed and the rooted plants were placed in soil in flats. After they were well established the plants were removed to the field. Seeds produced in 1953 were hand picked. In 1954 the number of culms per plant was considerably greater and each plant was cut, threshed, and the seed cleaned.

Seedlings from seed produced in 1953 from colchicine treated plants were planted in the greenhouse in the spring of 1954. These seedlings were transplanted to the field when well established. Spikes produced on these plants were harvested at maturity and the per cent of florets that set seed was calculated for each plant. One spike per plant was fixed in Carnoy's 6:3:1 for cytological observation.

Morphological Studies

Measurements of variation and estimates of population means for morphological characters were made from mass collections. A single culm from each selected plant was pressed and mounted for later study. Individual plants of *A. sericeum*, *A. repens*, and *E. canadensis* were selected at random while all plants of X*Agroelymus* encountered were selected. The number of specimens collected of *A. sericeum*, *A. repens*, *E. canadensis* and X*Agroelymus* were 54, 18, 47, and 37, respectively. Representative samples from the mass collections were deposited in the Iowa State College Herbarium and the remainder were deposited in the Herbarium of the Agronomy Department, Alaska Agricultural Experiment Station, Palmer, Alaska.

Measurements of floral parts were made with an eyepiece micrometer in a wide field binocular microscope. Data on dimensions of the glumes, lemmas, paleas and anthers were recorded in millimeters. A single spikelet was selected at random from each spike and dimensions of the glumes, lemma, palea and anthers were made on the lowermost floret of the spikelet. Data on length of culm, flag leaf blade and spike were recorded in centimeters. The number of nodes per culm, nodes per spike, and spikelets per node also were recorded. Means, ranges, and standard deviations for all characters were calculated. Descriptive notations also were recorded for the above and for several other organs.

Amino Acid Characterization

Tissues of *A. sericeum*, *A. repens*, *E. canadensis* and X*Agroelymus* were analyzed for their content of 13 amino acids. The following tissues were analyzed: (1) leaf blades which were harvested when the first growth was six-eight inches tall, (2) leaf blades which were harvested when plants were in the boot stage, (3) rhizomes collected in late autumn after the storage of reserves was completed, (4) mature dry seed, and (5) germinating seed.

Leaf blades were picked and promptly sealed in polyethelene bags, and frozen at -8° C. Rhizomes were picked from mature plants, washed in cold tap water, blotted with paper toweling and frozen. Seeds were germinated in petri dishes on four layers of paper toweling which had been saturated with a 0.2 per cent solution of potassium nitrate. Germination occurred under alternating temperatures of 20 and 30° C., as prescribed for several *Agropyrons* in U. S. Department of Agriculture Handbook 30 (41). Germination was halted by freezing when the coleoptile was one-fourth to one-half inch long. Freezing served to preserve tissues for analysis at more convenient times and facilitated homogenization by breaking down cell walls.

Leaves, rhizomes, and germinating seed were removed from the freezer and diced. Dry seed was ground to pass through a 40-mesh screen. Two and on-half grams of each tissue of each species was homogenized in 25 millileters of acetate buffer at a pH of 5.6. Following homogenization the liquid containing the free amino acids was decanted and centrifuged at approximately 18,000 times gravity to remove suspended materials such as plastids, mitochondria, other cellular fragments. The supernatant liquid was stored at 3.5° C.

Descending chromatographic analyses were made for free amino acid content according to the method of McFarren and Mills (19). Solvents used were phenol at pH 12.0 and m-cresol at pH 8.4. Chromatograms were developed at 21.0° C. $\pm 1^{\circ}$ C. Whatman No. 1 filter paper strips were air dried and dipped in a solution of 1.4 per cent ninhydrin in water-saturated n-butanol to which was added 4 per cent acetic acid for strips that were to be developed with phenol and 2 per cent acetic acid for strips developed with m-cresol. Strips were dried at 60° C. for 15 minutes. Each tissue test was made in duplicate.

Amino acids were identified by comparison of RF values to known acids and values as reported by McFarren and Mills (19). Quantitative values were obtained by measuring the per cent light transmitted through the amino acid spot with a densitometer. Curves were obtained for known amounts of various amino acids and the milligrams per gram of fresh weight of each amino acid in an unknown was determined from its respective curve.

EXPERIMENTAL RESULTS

Fertility Studies

Several hundred spikes were examined in 1951, 1952, 1953 and 1954 on *XAgroelymus* plants that had been transplanted to the breeding nursery in 1950 but no seeds were found. This emphasizes the complete sterility of this hybrid with regard to F_2 or backcrosses to either parent. Anthesis in the hybrid and parents overlapped and adequate opportunity for backcrossing existed.

Attempts to synthesize the hybrid through controlled pollinations in the greenhouse met with failure. Although over 100 pollinations were made, only two seeds were produced and these produced plants classified as selfed progeny of *E. canadensis*.

Similar results were obtained from attempts to produce the hybrid in the field with hot water emasculation. Eighty-one seeds from 12 spikes of *A. sericeum* and 95 seeds from nine spikes of *E. canadensis* were obtained. All plants derived from these seeds resulted from self-pollination. Nineteen emasculated but unpollinated spikes of *A. sericeum* produced 45 seeds and nine emasculated but unpollinated spikes of *E. canadensis* produced 46 seeds. *A. sericeum* produced an average of seven seeds per spike when emasculated and pollinated with E. canadensis pollen and two seeds per spike when emasculated but not pollinated. Corresponding values for E. canadensis were ten and five. E. canadensis spikes under bag which were neither emasculated nor pollinated produced 40 seeds per spike. The checks for A. sericeum were damaged in the field. Plants of both species grown under greenhouse isolation produced a high seed set which indicates high self-fertility.

Cytological Studies

A summary of observations on meiosis of A. sericeum, E. canadensis and XAgroelymus is given in Table 1. Meiotic stages are shown in Figures 1-45.

A. sericeum is a tetraploid with a somatic chromosome number of 28. No previous report of the chromosome number of this species was found in the literature. Meiosis is predominantly regular (Figures 1-8) although some irregularities occur (Figures 9-17). Usually 14 bivalents were observed at diplotene, diakinesis, and metaphase I (Figures 1, 2). A regular 14-14 disjunction is evident at anaphase I (Figure 3) and approximately 90 per cent of stainable pollen was found (Figure 8). This approximated the percentage of quartet cells without micronuclei.

Irregularities observed were chiefly univalents at

Table 1. Summary of observations concerning meiotic behavior of *A. sericeum*, *E. canadensis*, and X*Agroelymus*

Stage of meiosis	Classification	*A. sericeum* No. of sporocytes	*A. sericeum* % of total	*E. canadensis* No. of sporocytes	*E. canadensis* % of total	X*Agroelymus* No. of sporocytes	X*Agroelymus* % of total
Diplotene and diakinesis	14 bivalents	2	100	97	51	11	14
	Univ. or multivalents	0	0	0	0	42	53
	Undetermined	0	0	93	49	26	33
Metaphase I	14 bivalents	[a]		[a]		4	1
	Univ. or multivalents					306	80
	Undetermined					74	19
Anaphase I	Normal	146	70	40	91	16	24
	Bridges	19	10	4	9	21	31
	Laggards	43	20	0	0	30	45
Telophase I	Normal	169	86	193	99	31	42
	Exclusions	27	14	1	1	42	58
Quartet cells	Normal	553	88	1100	99	904	61
	Exclusions	72	12	10	1	583	39

[a] All determinable figures had 14 II. Most metaphase I figures were undeterminable in this material.

Figures 1-8. Regular meiosis in _A. sericeum_
(Figures 1-7 1000X, Figure 8 100X)

1 - Diplotene, 14II

2 - Metaphase I, 14II

3 - Anaphase I, 14-14 disjunction

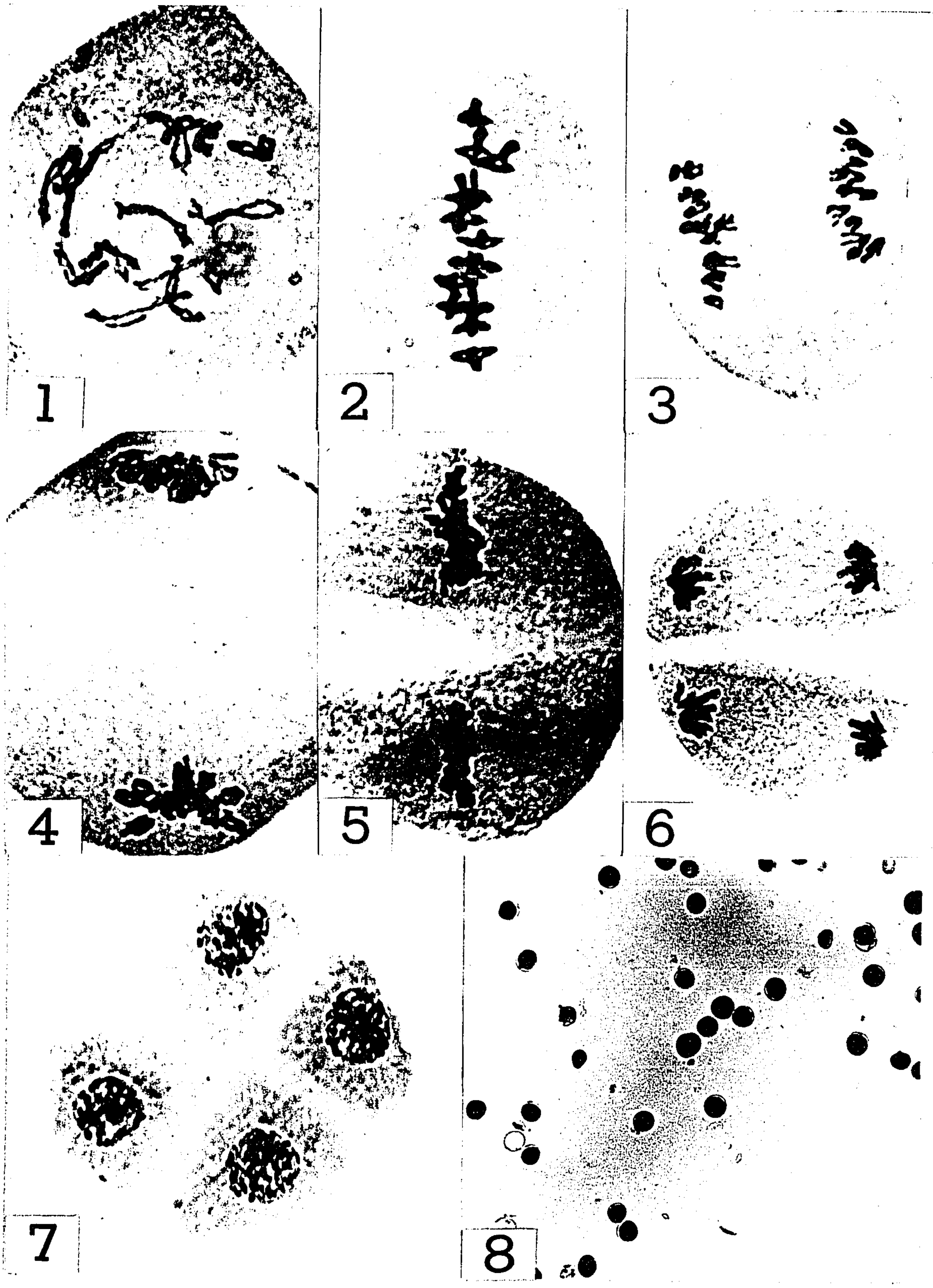
4 - Telophase I

5 - Metaphase II

6 - Telphase II

7 - Quartet, absence of micronuclei

8 - Pollen grains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Figures 9-17. Irregular meiosis in _A. sericeum_ (Figures 9-17 1000X)

9 - Metaphase I, univalent chromosomes

10 - Anaphase I, bridge, acentric fragment and lagging univalent

11 - Anaphase I, bridge

12 - Anaphase I, three bridges and acentric fragments

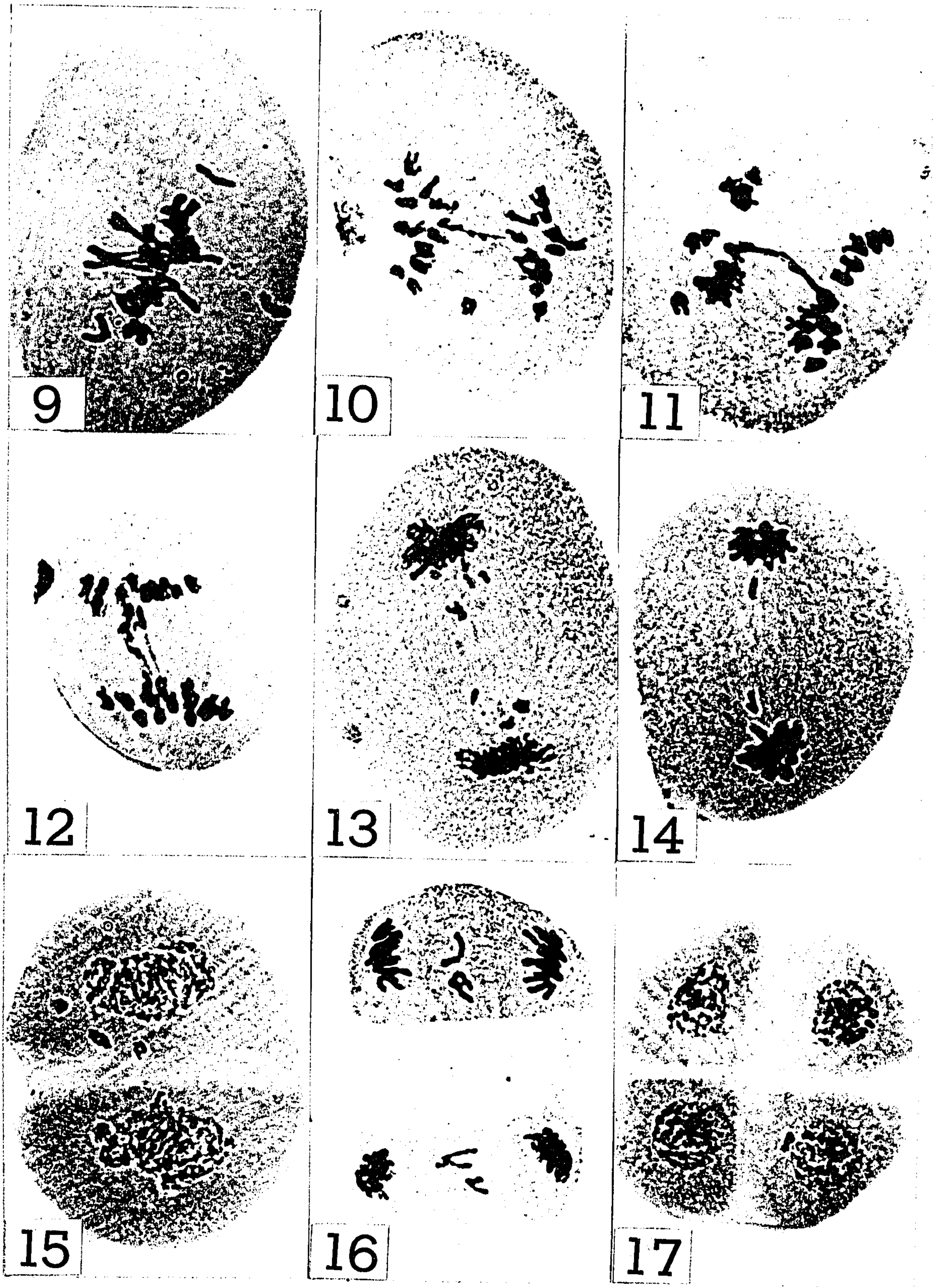
13 - Telophase I, three lagging univalents which have divided and are migrating poleward

14 - Telophase I, single lagging univalent

15 - Dyad, chromosomal exclusions

16 - Telophase II, lagging chromosomes

17 - Quartet, micronuclei in one cell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metaphase I (Figure 9) which appear to divide late resulting in laggards at anaphase I (Fig. 10) and telophase I (Figures 13-14). No correlations were made between frequency of univalents at metaphase I or laggards at anaphase I and quartet cells with micronuclei. Laggards were noted in about 20 per cent of the sporocytes examined. Bridges (Figures 10, 11, 12) at anaphase I resulting from chiasmata in heterozygous inversions were found in approximately 9 per cent of the sporocytes observed. Lagging chromosomes also were present at telophase II (Figure 16).

Meiosis in E. canadensis was very regular (Figures 18-27). Diplotene and diakinesis stages (Figure 18) were abundant and 97 of them contained 14 bivalents. Of those undetermined none clearly displayed either multivalents or univalents. A similar condition existed in metaphase I (Figure 19), although only a very few metaphases could be determined with certainty. In many sporocytes of this species a dark staining body was noted (Figure 18). Whether this was a supernumerary chromosome was not determined.

The only meiotic irregularities noted in this species were occasional bridges at anaphase I (Figure 22) and these occurred in 10 per cent of the figures. This probably was correlated with approximately 25 per cent of inviable pollen in the species (Figure 27). Intermediate staining pollen grains were considered viable. The reasons for variations

Figures 18-27. Meiosis in *E. canadensis*
(Figures 18-26 1000X, Figure 27 100X)

18 - Diplotene, 14 II and possible supernumerary chromosome at nine o'clock - light staining body at top is foreign material

19 - Metaphase I, 14 II

20 - Anaphase I, 14-14 disjunction

21 - Telophase I

22 - Telophase I, bridge

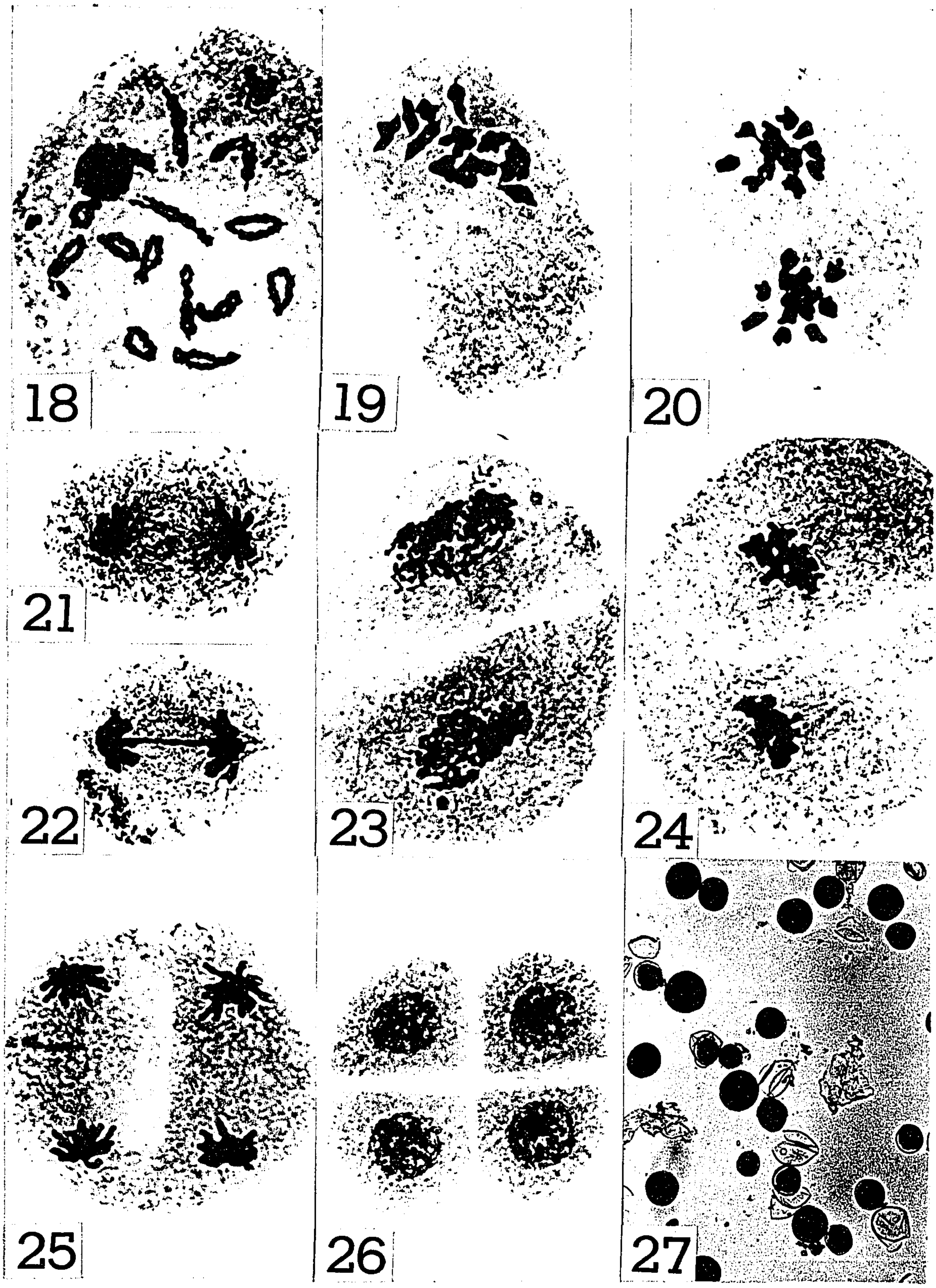
23 - Dyad

24 - Metaphase II

25 - Telophase II

26 - Quartet

27 - Pollen grains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in size and stainability of the pollen grains were not ascertained.

The meiosis of X*Agroelymus* was characterized by a high degree of irregularity (Figures 28-36) although normal figures for all stages were noted (Figures 37-45). Of 79 sporocytes observed in diplotene and diakinesis, 53 per cent definitely had univalents and/or multivalent associations. One-third of the 79 sporocytes were undetermined, although multivalent associations may have partially accounted for the undeterminability. Fourteen per cent of the sporocytes at diplotene and diakinesis had 14 bivalents. Only 1 per cent of metaphases were diagnosed as having 14 bivalents. Eighty per cent had either univalents or multivalents or both and 19 per cent were undetermined. Some multivalent associations were very complex (Figure 30) and probably involved more than four chromosomes. A number of sporocytes had a very high proportion of univalents (Figures 28, 29, 38) and these were found frequently in anthers of a plant that showed almost complete pairing in other sporocytes.

Approximately 24 per cent of the anaphase I figures were normal, 31 per cent had bridges and 45 per cent had lagging chromosomes. At telophase I, 58 per cent of the observed figures had exclusions of chromosomal material, whereas 42 per cent appeared normal. However, 61 per cent

Figures 28-36. Meiosis of *XAgroelymus* - irregular series (Figures 28-36 1000X)

28 - Diakinesis, polar view, 2 II and 24 I

29 - Metaphase I, polar view, 4 II and 20 I

30 - Metaphase I, multivalents and univalents

31 - Metaphase I, univalents

32 - Anaphase I, lagging chromosomes and remains of bridge

33 - Telophase I, bridge and lagging chromosomes

34 - Dyad, chromosomal exclusions

35 - Metaphase II, excluded bodies from first division

36 - Quartet with micronuclei

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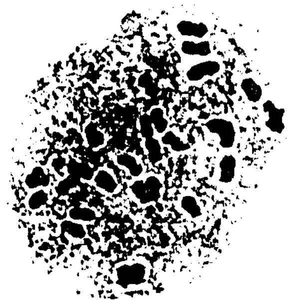

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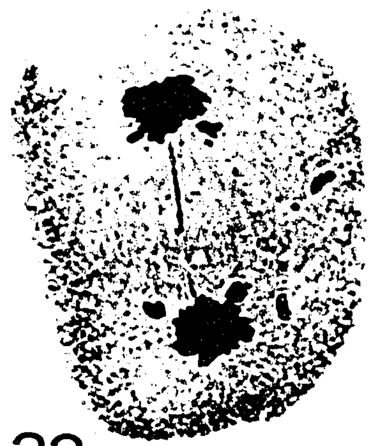

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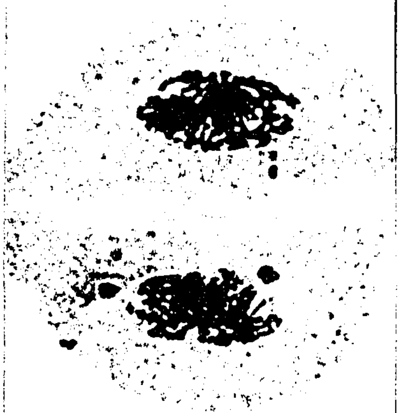

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Figures 37-45. Meiosis in *XAgroelymus* - regular series (Figures 37-44 1000X, Figure 45 100X)

37 - Diakinesis, polar view, 14 II

38 - Metaphase I, 2 II and 24 I

39 - Anaphase I, normal disjunction

40 - Telophase I

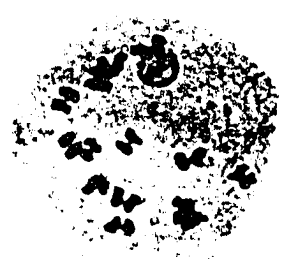
41 - Dyad

42 - Metaphase II

43 - Telophase II

44 - Quartet without micronuclei

45 - Pollen, completely sterile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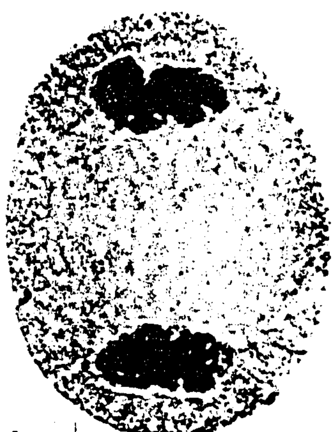
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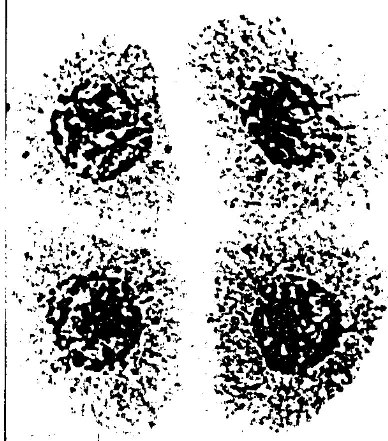
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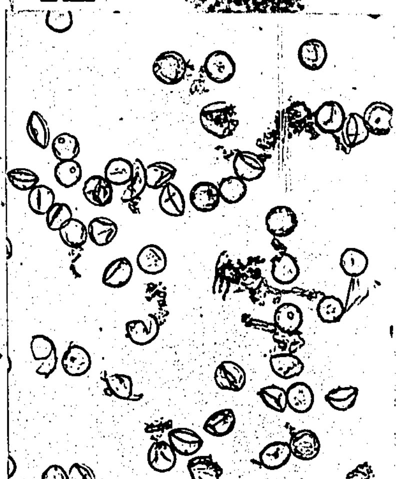
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of the quartet cells were devoid of micronuclei. Pollen in XAgroelymus was completely sterile (Figure 45).

In 1953 XAgroelymus plants that developed from colchicine treated sections were examined for production of seed. Twenty seeds were found on about 250 plants. In 1954 the colchicine treated plants were examined again. Individual plants were threshed and the seed separated from the chaff. More than 40 grams of seed was obtained.

Seed produced in 1953 was germinated in moist vermiculite at alternating temperatures of 20 and 30° C. Seedlings were transplanted to soil in the greenhouse and removed to the field in late May. Fertility of these plants, calculated as the per cent of florets containing a caryopsis, ranged from 0.22 to 20.0 with a mean of 8.45. Nineteen plants produced 225 spikes, 19,309 florets and 1,631 seeds.

The few aceto-carmine smears that were made indicated considerable irregularity and apparently an octoploid chromosome complement.

Morphological Studies

Gross morphology of the spikes of A. sericeum, A. repens, E. canadensis and XAgroelymus is depicted in Figure 46. On gross morphological grounds E. canadensis was one parent of the hybrid and either Agropyron species could

Figure 46. Comparison of gross morphology of spikes, left to right, of *A. sericeum*, X*Agroelymus*, *E. canadensis*, and *A. repens*

have been the other. The length of spike, however, favored *A. sericeum*. Figure 47 shows an entire plant of X*Agroelymus* and emphasizes its vigor, leafiness, and abundance of flowering culms. In the breeding nursery the hybrid began growth earlier in spring than any of the other species and also reached a grazing height much earlier. Its vigor and early spring growth are characters of considerable value from the standpoint of its potential use for grazing.

Measurements of floral organs of the four grasses were made and mean values, standard deviations and ranges for measurements of lemmas, paleas, glumes and anthers are given in Table 2. With respect to length of lemmas, paleas and anthers X*Agroelymus* was much closer to *A. sericeum* than *A. repens*. In fact, the shortest anther of *A. repens* was more than double the length of the longest of the remaining three grasses. Only in the case of length of glume awn was the hybrid nearer to *A. repens*.

Regarding floral characteristics, the polygons for *A. sericeum*, *E. canadénsis*, and X*Agroelymus* (Figure 48) are considerably more congruous than those for *A. repens*, *E. canadensis*, and X*Agroelymus* (Figure 49) and on this basis *A. sericeum* is more likely to be the *Agropyron* parent than is *A. repens*.

The ideograph in Figure 50 shows the relation of lemma length and width to glume length and width and illustrates

Figure 47. *XAgroelymus* plant showing pronounced vigor, leafiness, and abundance of flowering culms - gridded background is 4 feet square

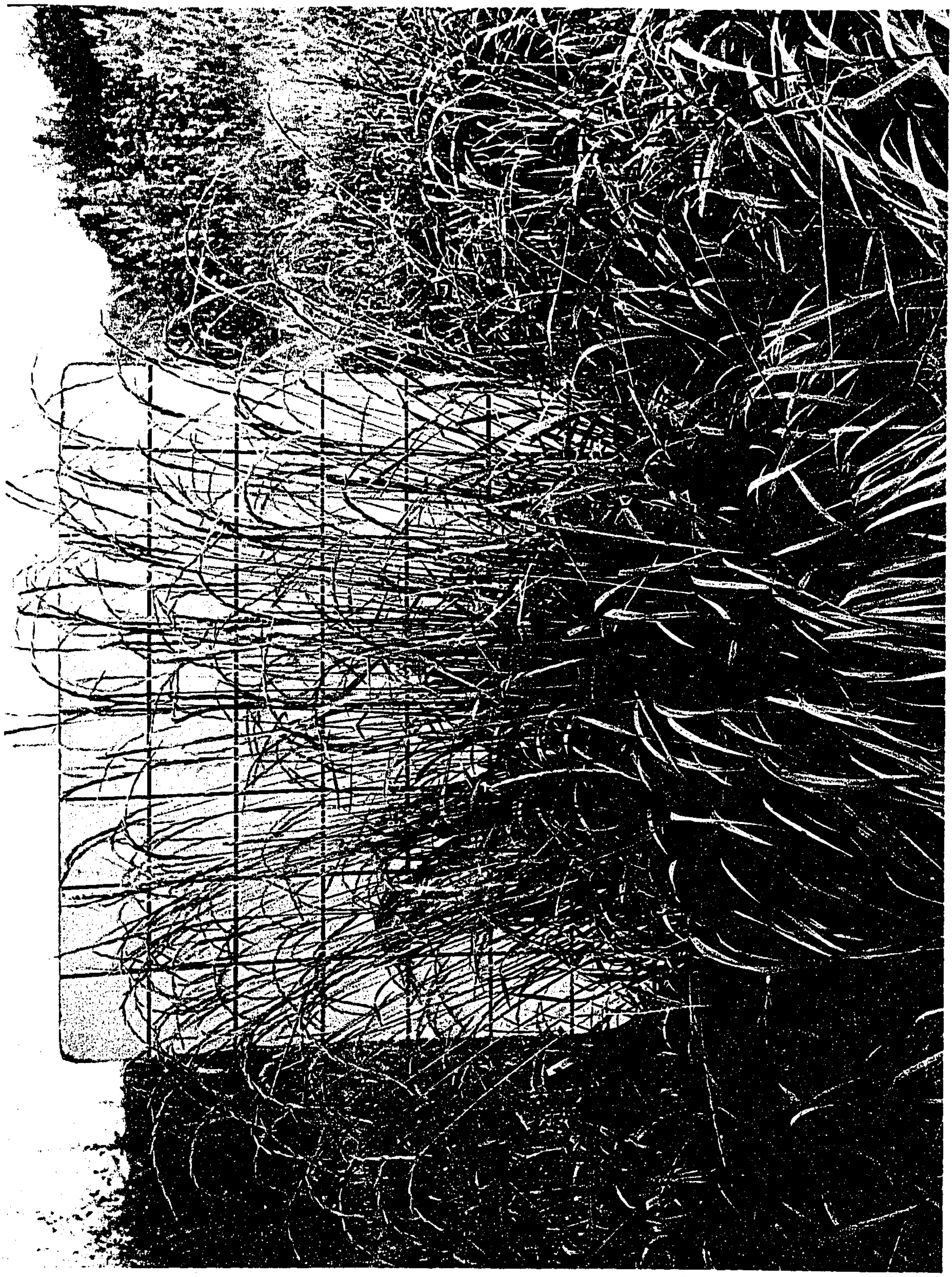


Table 2. Mean dimensions with standard deviations and ranges for various floral organs of four grasses (measurements in mm.)

Character	*A. sericeum* Mean ± S.D.	*A. repens* Mean ± S.D.	*E. canadensis* Mean ± S.D.	*XAgroelymus* Mean ± S.D.
Lemma length	11.2 ± .8	9.2 ± .6	12.2 ± 1.2	12.3 ± .9
range	9.1 - 12.5	8.0 - 10.5	9.5 - 14.2	10.0 - 14.6
width	1.8 ± .2	1.6 ± .2	1.8 ± .2	1.8 ± .2
range	104.0 - 2.1	1.2 - 2.0	1.3 - 2.2	1.3 - 2.4
awn	0.7 ± .3	1.0 ± 1.1	20.3 ± 4.0	7.6 ± 1.9
range	0.0 - 1.5	0.0 - 3.5	11.5 - 31.0	4.5 - 13.0
Palea length	11.1 ± .8	8.2 ± .6	11.7 ± 1.1	12.3 ± .9
range	9.0 - 12.5	7.1 - 9.5	9.4 - 14.6	10.0 - 14.5
width	1.4 ± .1	1.3 ± .2	1.1 ± .1	1.2 ± .2
range	1.1 - 1.7	1.0 - 1.7	0.7 - 1.5	0.9 - 1.4
Glume length	7.6 ± .8	7.5 ± 1.1	5.8 ± 1.0	7.1 ± .9
range	6.0 - 9.0	5.1 - 11.0	4.1 - 8.0	5.2 - 10.0
width	1.3 ± .2	1.1 ± .2	0.7 ± .2	1.1 ± .1
range	0.7 - 1.8	0.8 - 1.6	0.5 - 1.0	0.7 - 1.7
awn	0.5 ± .3	1.3 ± .6	3.0 ± 1.3	1.3 ± .2
range	0.0 - 1.1	0.1 - 1.7	0.5 - 7.0	0.5 - 3.1
Anther length	1.8 ± .1	5.1 ± .5	1.7 ± .1	1.6 ± .2
range	1.5 - 1.9	4.2 - 6.2	1.4 - 1.9	1.3 - 2.0

Figure 48. Polygonal graph depicting means of six floral characters of _A. sericeum_, _E. canadensis_, and X_Agroelymus_ (measurements in mm.)

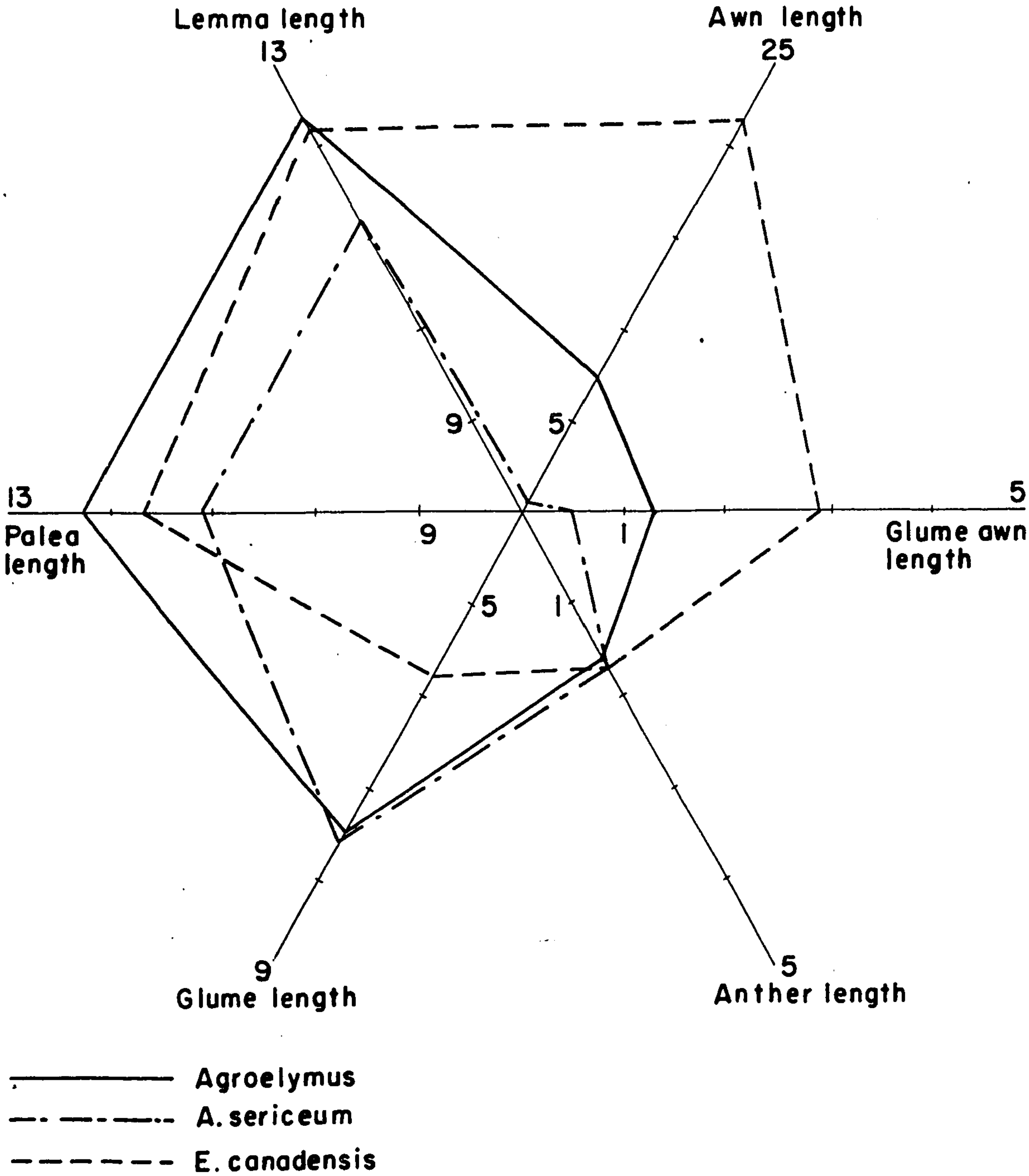

Lemma length
13
Awn length
25
9
5
13
Palea length
9
1
5
Glume awn length
5
1
9
Glume length
5
Anther length
Agroelymus
A. sericeum
E. canadensis

Figure 49. Polygonal graph depicting means of six floral characters of *A. repens*, *E. canadensis*, and X*Agroelymus* (measurements in mm.)

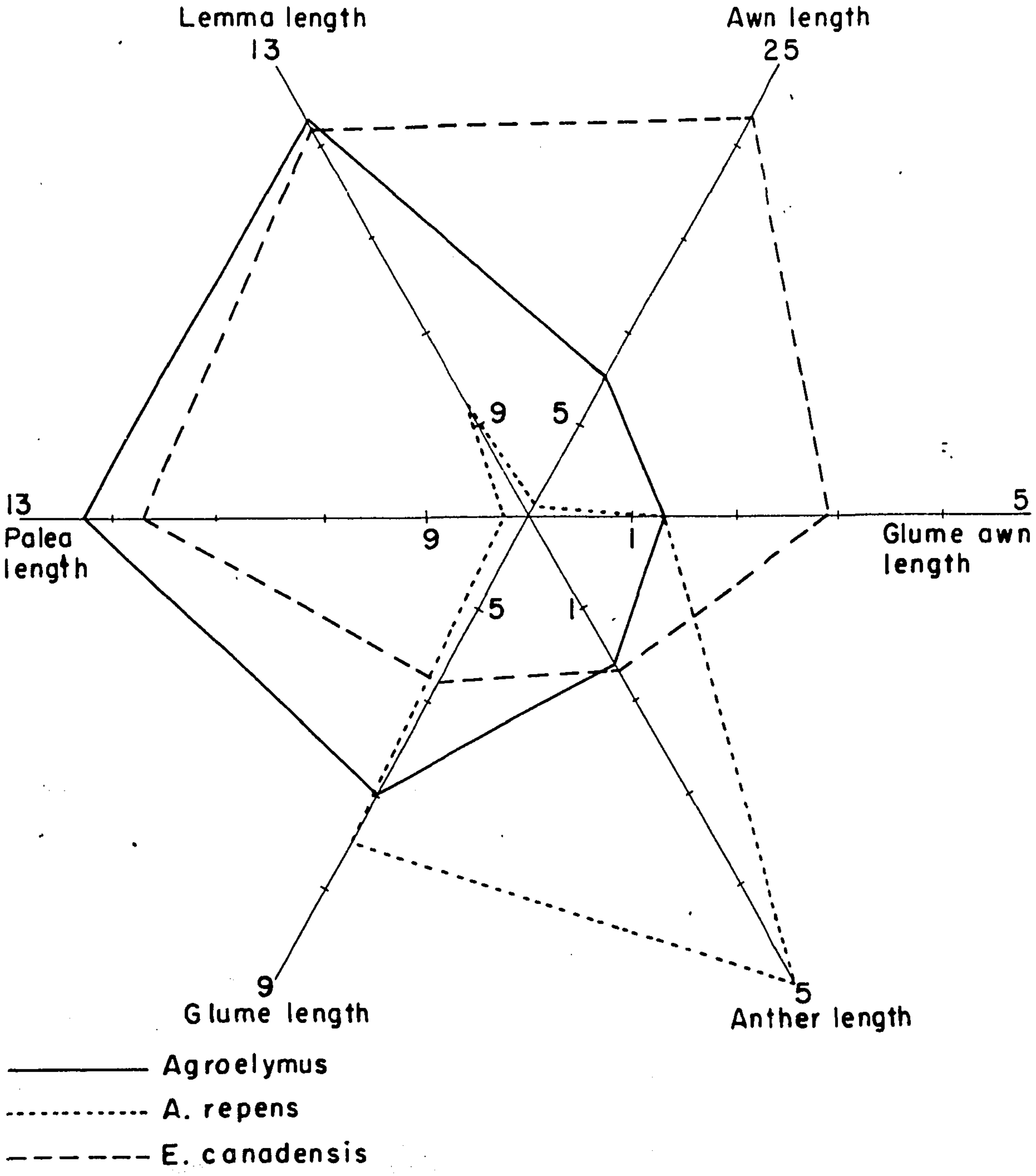
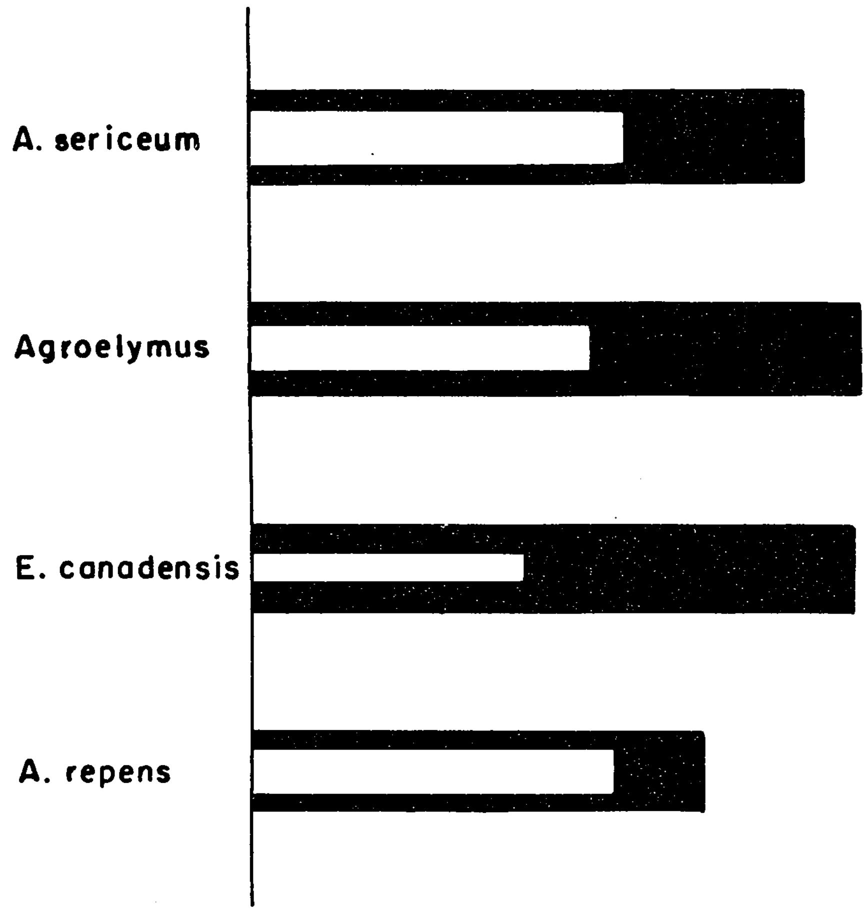

Lemma length
13
Awn length
25
9
5
13
Palea length
9
1
5
Glume awn length
5
1
9
Glume length
5
Anther length
Agroelymus
A. repens
E. canadensis

Figure 50. Ideograph showing relation of lemma length and width (shaded) to glume length and width (measurements in mm.)



A. sericeum
Agroelymus
E. canadensis
A. repens

further that XAgroelymus is more nearly intermediate between E. canadensis and A. sericeum than E. canadensis and A. repens.

Gross morphology of the spikelets is shown in Figure 51 and details of the lemma, glumes and rachillae are shown in Figure 52. With respect to spikelet morphology as well as the characteristics of the lemmas, glumes and rachillae, XAgroelymus is a clear intermediate between A. sericeum and E. canadensis. It is very unlikely that the glumes and rachillae of XAgroelymus resulted from a combination of A. repens and E. canadensis.

Comparisons of measurements of vegetative organs of the four grasses are given in Table 3. Culm length and spike length exhibit heterosis and are of little value in delineating parentage of the hybrid. Length of flag leaf blade, nodes per culm, and nodes per spike indicate that A. sericeum is the more likely Agropyron parent.

The polygons for the various grasses in Figures 53 and 54 also illustrate a greater similarity between XAgroelymus and A. sericeum than A. repens and the histogram in Figure 55 depicts a similar relationship.

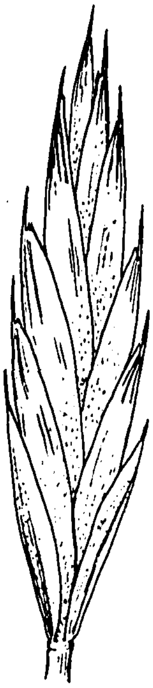
Table 4 contains descriptive data for various characteristics of the four grasses in question and Figure 52 illustrates some of these characteristics. A. repens is very strongly rhizomatous while all others are more or less

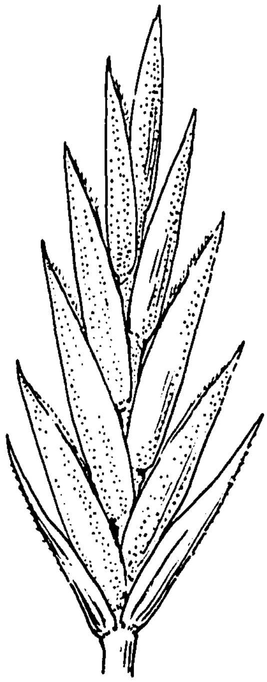
Figure 51. Upper left - spikelet of *Agropyron sericeum* X3

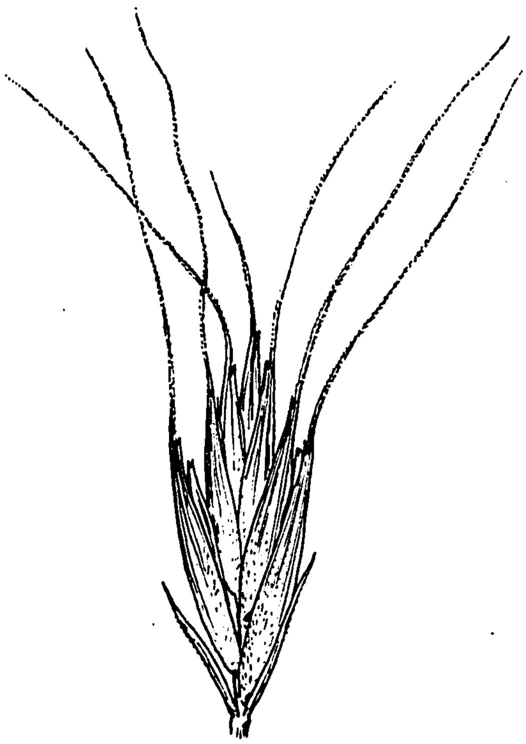
Upper right - spikelet of *Agropyron repens* X4

Lower left - spikelet of *Elymus canadensis* X2

Lower right - spikelet of X*Agroelymus* X3







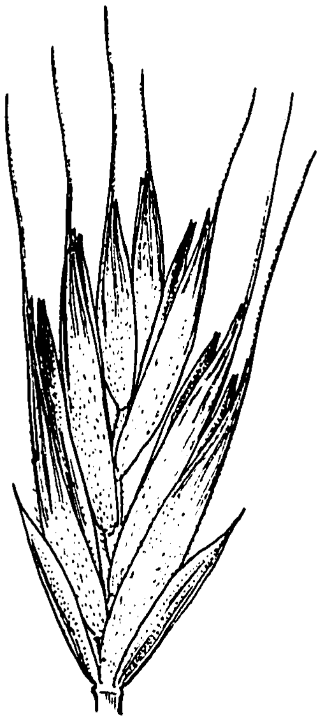


Figure 52. Top row, left to right - lemmas of _Agropyron sericeum_, _Agropyron repens_, _Elymus canadensis_, and X_Agroelymus_ X5

Center row, left to right - glumes of _Agropyron sericeum_, _Agropyron repens_, _Elymus canadensis_, and X_Agroelymus_ X6

Bottom row, left to right - Rachillae of _Agropyron sericeum_, _Agropyron repens_, _Elymus canadensis_, and X_Agroelymus_ X15

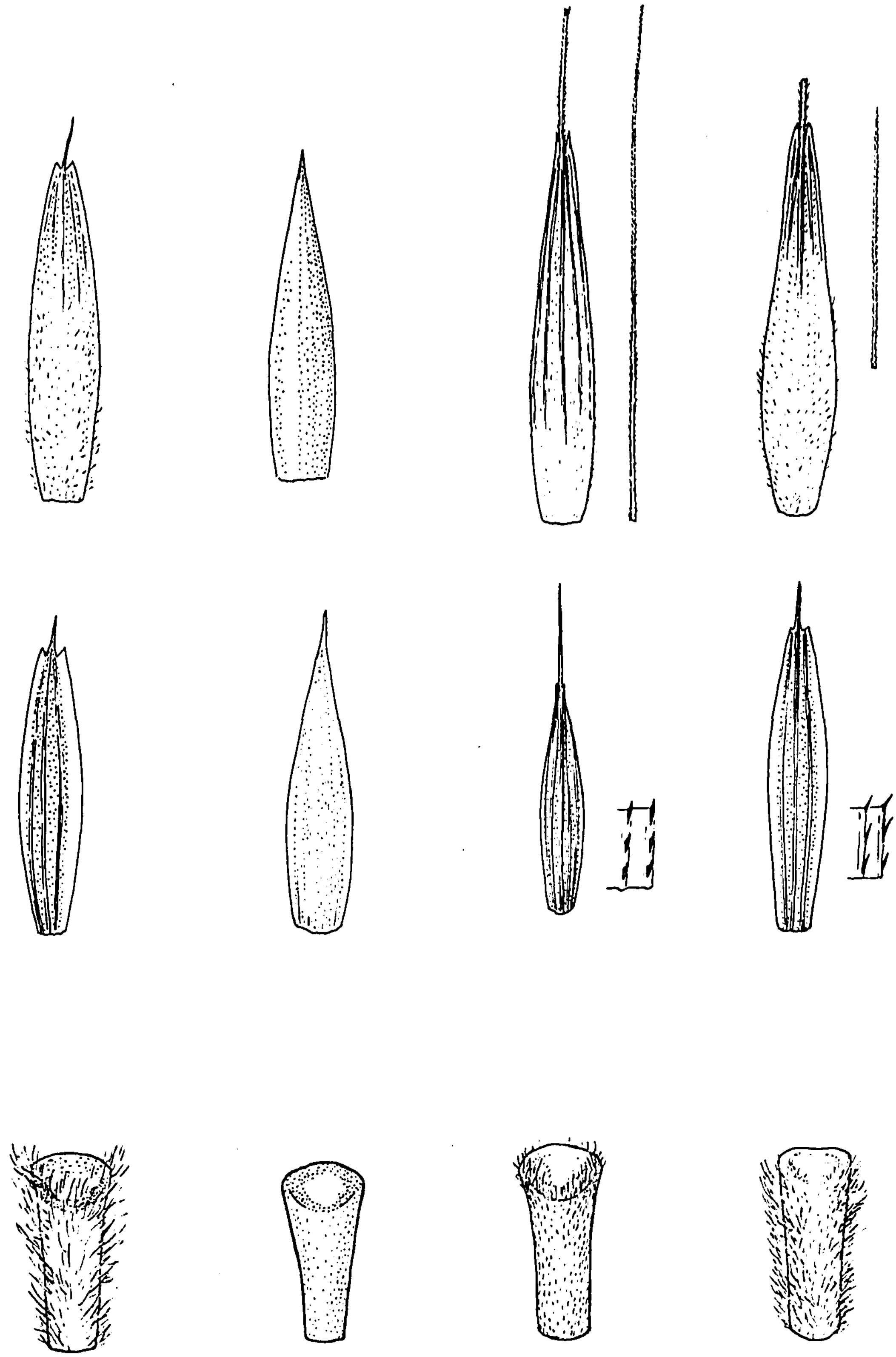
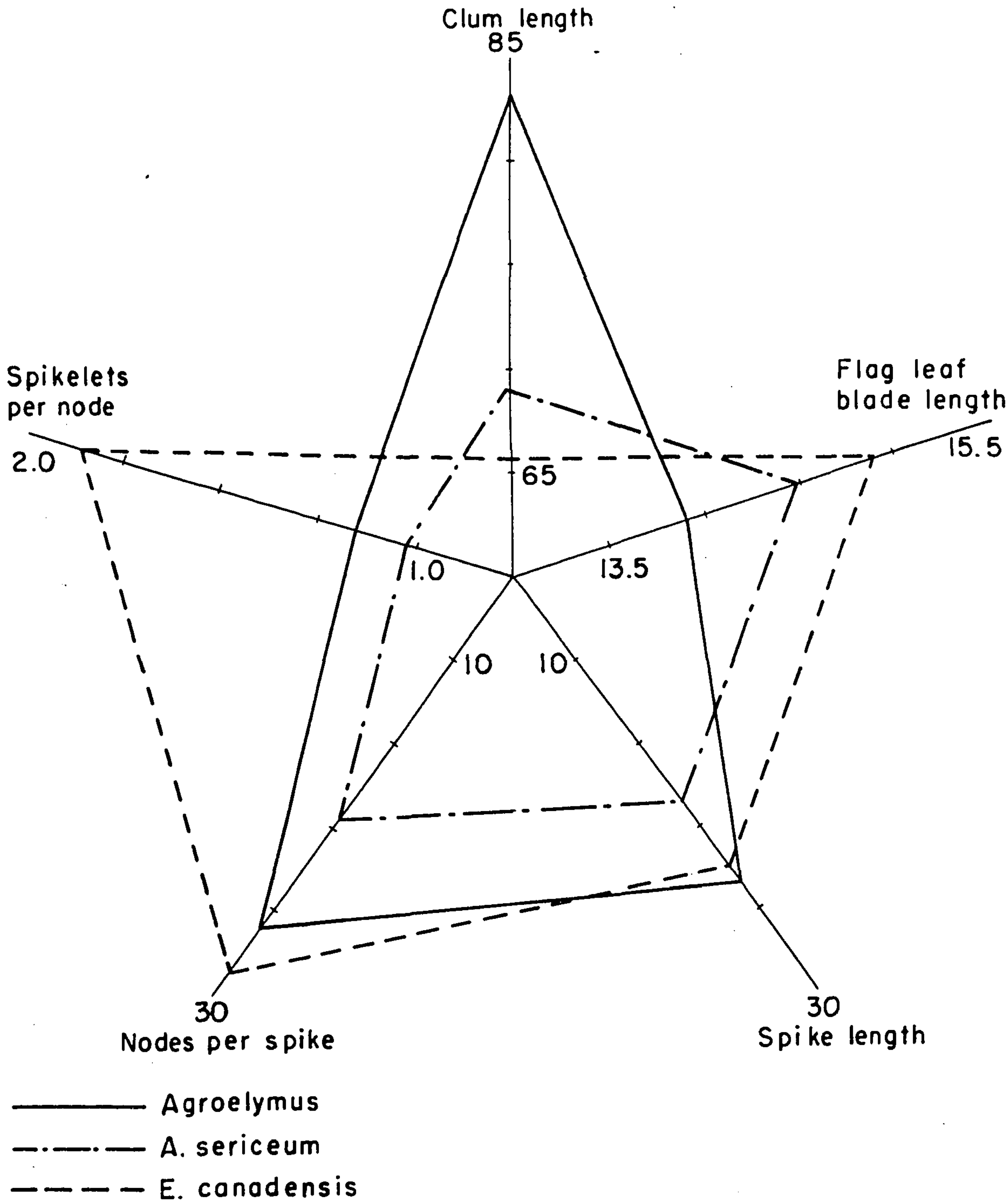


Table 3. Mean dimensions with standard deviations and ranges for vegetative organs of four grasses (measurements in cm.)

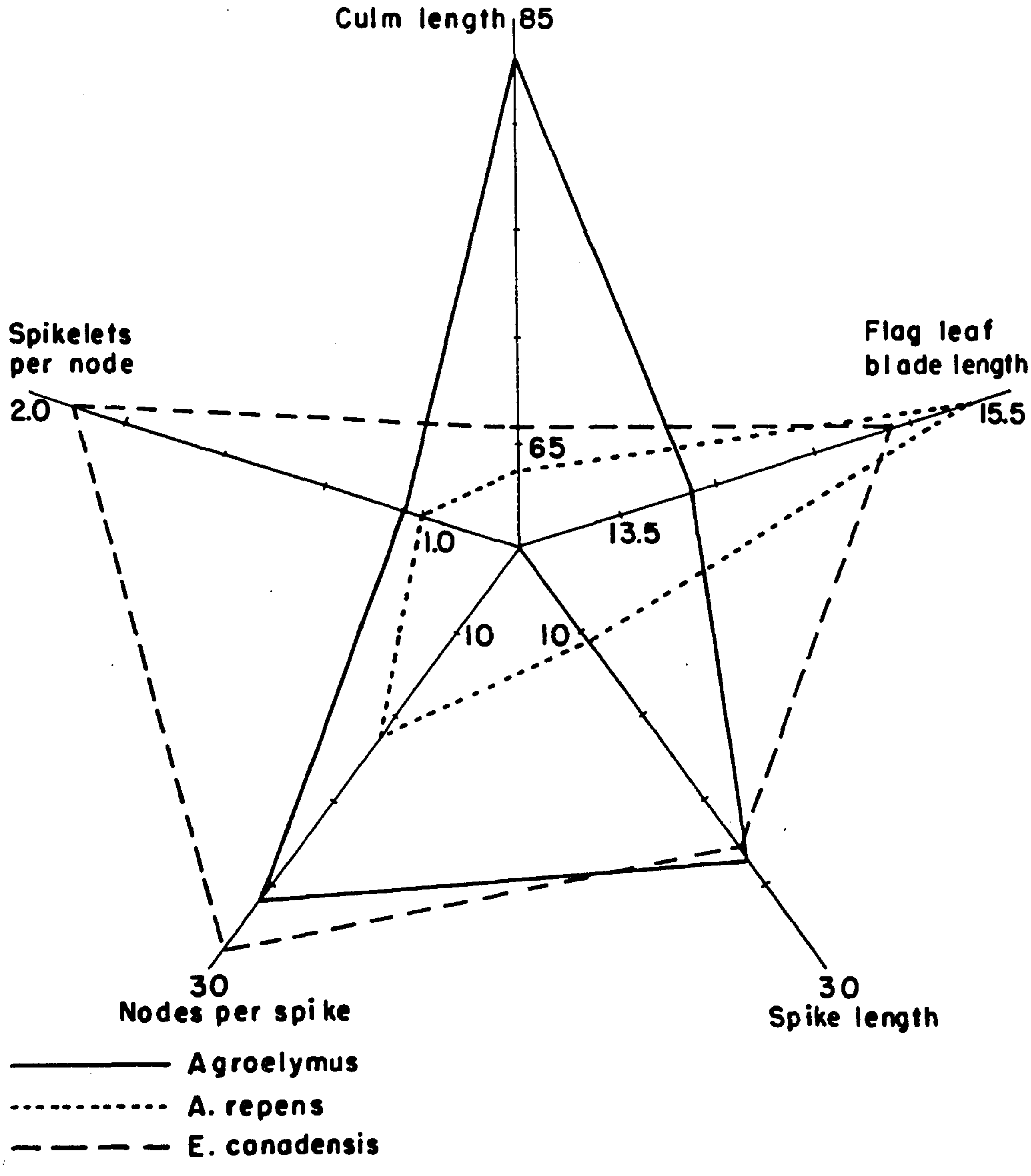
Character	*A. sericeum*	*A. repens*	*E. canadensis*	*XAgroelymus*
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
Culm length	69.8 ± 9.6	63.4 ± 7.1	66.0 ± 11.7	83.2 ± 9.8
range	55.2 – 96.9	55.7 – 75.1	42.2 – 98.4	61.6 – 101.7
Flag leaf blade length	14.5 ± 4.0	15.3 ± 3.3	14.9 ± 4.2	13.9 ± 4.3
range	4.8 – 25.0	8.5 – 20.4	7.3 – 23.0	5.0 – 26.0
Spike length	18.7 ± 3.0	10.4 ± 1.9	22.6 ± 3.2	23.4 ± 4.3
range	11.8 – 27.3	7.2 – 12.7	14.8 – 29.0	12.7 – 37.5
Nodes per spike	19.6 ± 3.4	16.2 ± 1.8	28.7 ± 3.8	26.0 ± 3.6
range	11.0 – 27.0	13.0 – 19.0	20.0 – 36.0	20.0 – 33.0
Spikelets per node	1.0	1.0	1.9	1.2
Nodes per culm	3.0	4.4	3.8	3.8

Figure 53. Polygonal graph depicting means of five vegetative characters of *A. sericeum*, *E. canadensis*, and X*Agroelymus* (measurements in cm.)



Clum length
85
65
Flag leaf
blade length
15.5
13.5
Spikelets
per node
2.0
1.0
10
10
30
Nodes per spike
30
Spike length
Agroelymus
A. sericeum
E. canadensis

Figure 54. Polygonal graph depicting means of five vegetative characters of _A. repens_, _E. canadensis_, and X_Agroelymus_ (measurements in cm.)



Culm length 85
65
Flag leaf blade length
15.5
13.5
Spikelets per node
2.0
1.0
10
10
30
Nodes per spike
30
Spike length
Agroelymus
A. repens
E. canadensis

Figure 55. Relation of length of culm to length of spike (measurements in cm.)

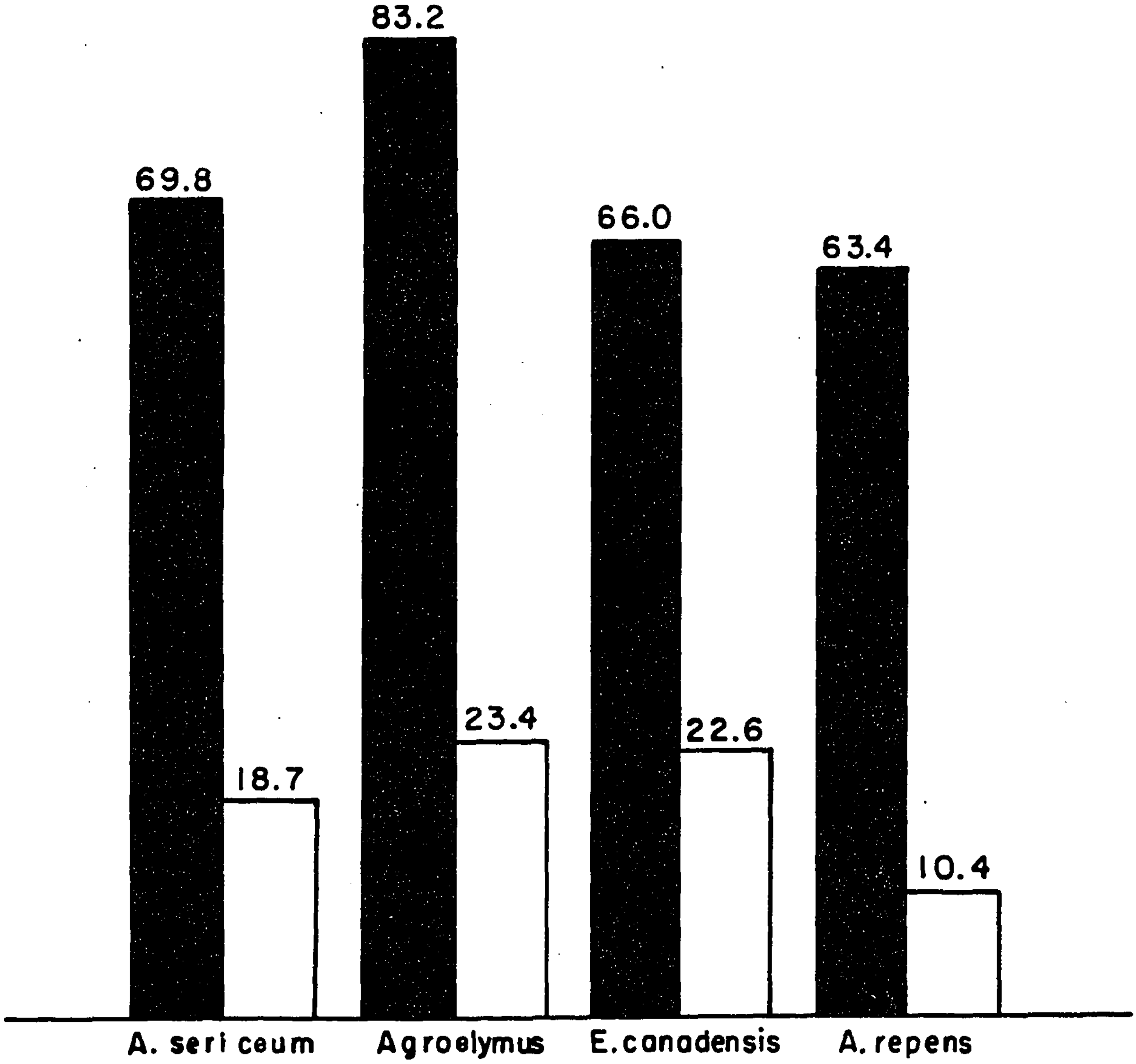

83.2
69.8
66.0
63.4
23.4
22.6
18.7
10.4
A. sericeum
Agroelymus
E. canadensis
A. repens

Table 4. Descriptive data for various characters in four grasses

Character	*A. sericeum*	*A. repens*	*E. canadensis*	*XAgroelymus*
Rhizomes	Cespitose (short ascending rhizome)	Long rhizomes	Cespitose (short ascending rhizome)	Cespitose (short ascending rhizome)
Leaves	5-12 mm. wide Scabrous above Scaberulous below	6-10 mm. wide Glabrous-scabrous on margins	5-12 mm. wide Scabrous both sides	5-10 mm. wide Scabrous both sides
Auricles	Absent or rare	Present	Absent or rare	Absent or rare
Spike	Erect 12-27 cm. long 4-8 mm. diameter	Erect 7-13 cm. long 8-12 mm. diameter	Pendant 15-29 cm. long 10-20 mm. diameter	Nodding 13-38 cm. long 8-15 mm. diameter
Rachis	Scabrous on edges	Scabrous on edges	Hispid on edges	Scabrous to hispid on edges
Spikelets	7-9 florets One per node	4-6 (8) florets One per node	5-7 florets 1-3 per node	7-9 florets 1-2 per node
Glumes	Wide scarious margin	Narrow scarious margin	Very narrow scarious margin	Intermediate scarious margin
	Oblong	Lanceolate-acuminate	Linear-acuminate	Lanceolate
	3-4 nerves	4-5 nerves	2-5 nerves	3 nerves
	Scabrous-sometimes slight pubescence at base	Glabrous	Very glabrous	Scabrous

Table 4. (Continued)

Character	*A. sericeum*	*A. repens*	*E. canadensis*	*XAgroelymus*
Lemma	5 nerves	Nerves obscure	5 nerves	5 nerves
	Weakly nerved at apex		Strongly nerved upper 2/3	Strongly nerved upper 1/3
	Strongly pubesent	Glabrous	Short pubescent at base and on sides	Moderately pubescent
Palea	Truncate	Truncate	Toothed	Truncate
	Strongly scabrous edges	Scabrous on edges	Weakly scabrous edges	Strongly scabrous edges
Rachilla	Strong pubescent	Glabrous or scaberulous	Short pubescent	Strong pubescent

cespitose. Auricles are nearly always present on this species and absent or rarely present on the others. In addition, leaves usually are glabrous or at most scabrous on the margins in _A. repens_ and scabrous on the remaining species. A similar comparison exists with regard to the glumes.

The glumes of X_Agroelymus_ have a wider scarious margin than those of any other species except _A. sericeum_ in which case the scarious margin ranges up to about one-third the width of the glume. The glumes of _A. repens_ generally have a larger number of nerves than the other species. Lemmas of _A. repens_ are glabrous while those of the remaining three species are pubescent in varying degrees. Finally, the rachillas of all except _A. repens_ are pubescent.

The comparisons show clearly that _A. repens_ differs considerably from _A. sericeum_, _E. canadensis_, and X_Agroelymus_ and provide strong evidence that _A. sericeum_ and not _A. repens_ is involved in the parentage of the X_Agroelymus_ in this study.

Amino Acid Characterization

The purpose of amino acid characterization was to determine whether possible differences in amino acid content between species may aid in determining the parentage of the hybrid. The presence or absence of 13 amino acids in five types of tissue of the four grasses is depicted in Table 5.

In seven instances, the four species differed with respect to the presence of amino acids. In each instance *A. repens* was deficient in an amino acid present in the remaining three species. Not all amino acids known to occur in plants are represented in this table and it is possible that other differences may exist either in the same direction or otherwise. However, these seven instances offer considerable indirect evidence that *A. sericeum* rather than *A. repens* is involved in the parentage of this hybrid.

Table 6 shows milligrams per gram of fresh weight of nine amino acids in the various tissues. From these data it appears impossible to make further inferences regarding the *Agropyron* parentage of the hybrid. There appeared to be an indication of heterosis with respect to total content of these nine amino acids in leaves and mature seed whereas in rhizomes and germinating seed the hybrid was intermediate between *E. canadensis* and both *Agropyron* species. The blades of young leaves had the highest amino acid content. Leaves harvested at the boot stage were somewhat lower, as was expected. Storage tissues, rhizomes, and mature seed were still lower and germinating seed was lowest by a considerable amount. It was expected that germinating seed would be higher in total amino acids than mature seeds or rhizomes in view of the presumed high rate of physiologic

Table 5. Presence (+) or absence (-) of amino acids in various tissues of four grasses
analysis

Species	Aspartic acid	Glutamic acid	Serine	Threonine	Alanine	Glycine	Unknown	Unknown
	Phenol as solvent							
					Leaf blades at 6-8 inch height			
A. sericeum	+	+	+	+	+	-	+	-
A. repens	+	+	+	+	+	-	+	-
E. canadensis	+	+	+	+	+	-	+	-
XAgroelymus	+	+	+	+	+	-	+	-
					Leaf blades at boot stage			
A. sericeum	+	+	+	+	+	-	+	-
A. repens	+	+	+	+	+	-	+	-
E. canadensis	+	+	+	+	+	-	+	-
XAgroelymus	+	+	+	+	+	-	+	-
					Rhizomes			
A. sericeum	+	+	-	+	+	-	+	+
A. repens	+	+	-	(-)	+	-	+	+
E. canadensis	+	+	-	+	+	-	+	+
XAgroelymus	+	+	-	+	+	-	+	+
					Mature dry seed			
A. sericeum	+	+	+	+	+	-	-	-
A. repens	+	+	+	+	+	-	-	-
E. canadensis	+	+	+	+	+	-	-	-
XAgroelymus [a]	+	+	+	+	+	-	-	-
					Germinating seed			
A. sericeum	-	-	+	+	+	+	-	-
A. repens	-	-	+	+	+	+	-	-
E. canadensis	-	-	+	+	+	+	-	-
XAgroelymus [a]	-	-	+	+	+	+	-	-

( ) Symbols in parentheses indicate instances where differences between grasses exist.
[a] Amphidiploid seed produced on colchicine treated F_1 plants.

acids in various tissues of four grasses as determined by descending chromatographic

s solvent					M-cresol as solvent				
ne	Alanine	Glycine	Unknown	Unknown	Histidine	Valine	Leucine	Hydroxy-proline	Methionine
	Leaf blades at 6-8 inch height								
	+	-	+	-	+	+	+	-	-
	+	-	+	-	+	+	(-)	-	-
	+	-	+	-	+	+	+	-	-
	+	-	+	-	+	+	+	-	-
	Leaf blades at boot stage								
	+	-	+	-	+	+	+	-	-
	+	-	+	-	+	+	+	-	-
	+	-	+	-	+	+	+	-	-
	+	-	+	-	+	+	+	-	-
	Rhizomes								
	+	-	+	+	+	+	+	-	-
	+	-	+	+	+	+	(-)	-	-
	+	-	+	+	+	+	+	-	-
	+	-	+	+	+	+	+	-	-
	Mature dry seed								
	+	-	-	-	+	+	+	+	+
	+	-	-	-	+	+	+	(-)	(-)
	+	-	-	-	+	+	+	+	+
	+	-	-	-	+	+	+	+	+
	Germinating seed								
	+	+	-	-	+	+	+	-	-
	+	+	-	-	+	(-)	(-)	-	-
	+	+	-	-	+	+	+	-	-
	+	+	-	-	+	+	+	-	-

here differences between grasses exist.
'eated F_1 plants.

Table 6. Milligrams per gram of fresh weight of nine amino acids in tissues of four grasses

	Phenol as solvent						M-cresol as solvent			
Species	Aspartic acid	Glutamic acid	Serine	Threonine	Alanine	Glycine	Histidine[a]	Valine	Methionine	Total
				Leaf blades at 6-8 inch height						
A. sericeum	6.5	3.5	1.0	11.5	6.4	-	43.0	3.2	-	75.1
A. repens	0.8	2.7	0.9	15.0	8.2	-	37.0	0.8	-	65.4
E. canadensis	2.0	2.1	1.0	15.3	7.8	-	32.0	1.0	-	61.2
XAgroelymus	0.8	2.1	0.9	15.9	6.4	-	54.0	2.3	-	82.4
				Leaf blades at boot stage						
A. sericeum	2.0	5.4	1.4	8.4	6.4	-	21.0	1.2	-	45.8
A. repens	0.7	2.8	0.9	7.1	5.2	-	40.0	1.7	-	58.4
E. canadensis	0.4	1.3	0.5	3.9	3.7	-	37.0	1.4	-	38.2
XAgroelymus	1.0	2.9	0.5	7.7	8.0	-	40.0	1.3	-	61.4
				Rhizomes						
A. sericeum	0.7	1.9	-	2.0	16.2	-	19.0	1.6	-	41.4
A. repens	0.7	1.5	-	-	16.2	-	26.0	0.4	-	44.8
E. canadensis	0.7	1.7	-	1.9	14.4	-	42.0	2.2	-	62.9
XAgroelymus	0.7	2.1	-	2.1	14.0	-	29.0	3.4	-	51.3
				Mature seed						
A. sericeum	1.2	2.6	0.4	0.9	8.3	-	26.0	3.8	1.5	44.6
A. repens	0.5	1.2	0.2	0.8	4.0	-	15.0	1.1	-	22.8
E. canadensis	1.0	2.1	0.5	1.8	7.0	-	24.0	3.5	1.3	41.2
XAgroelymus	2.0	2.3	0.7	1.5	8.3	-	37.0	3.1	1.2	57.1
				Germinating seed						
A. sericeum	-	-	0.5	0.3	5.2	0.4	3.0	0.5	-	9.9
A. repens	-	-	0.3	0.6	3.4	0.4	2.0	-	-	6.7
E. canadensis	-	-	0.5	0.5	3.4	0.5	9.0	0.7	-	14.2
XAgroelymus	-	-	0.4	0.3	4.3	0.4	5.0	0.5	-	10.9

[a]Values in excess of 18.0 milligrams per gram of fresh tissue were obtained by extrapolation of curves and thus are regarded only as approximate.

activity.

The hybrid was considerably higher than the parents in total amino acids in leaf tissue. This is of particular interest in view of the forage possibilities of this hybrid.

DISCUSSION

Failure of X*Agroelymus* to produce any seed, either F_2 or backcross, clearly illustrates the high degree of sterility of this hybrid and strongly indicates that it is a hybrid between two genetically isolated species. Strong circumstantial evidence is presented in this report that X*Agroelymus* is a hybrid between *Agropyron sericeum* and *Elymus canadensis* and thus corresponds to X*Agroelymus palmerensis* described by Lepage (16, 17). None of the specimens contained in the mass collection corresponds to X*Agroelymus Hodgsonii* described by Lepage in the same study as a hybrid between *Agropyron repens* and *E. canadensis*. Further, the isotype of X*Agroelymus Hodgsonii* (Lepage Collection No. 25,264) is very similar in general appearance to X*Agroelymus palmerensis* described herein. The pubescent rachilla and lemma, the narrow scarious margin of the glume, and length of anthers are identical with the specimens in the mass collection in this study.

The precise delineation of the parentage of X*Agroelymus* in this study must await the synthesis of the hybrid under controlled conditions. Although this has not yet been accomplished, it cannot be concluded that it is impossible. It is the opinion of the writer that more concentrated effort and more varied techniques would be successful.

Emasculation without injury to the female reproductive tissues was difficult because it was necessary to emasculate at a comparatively early stage in spike development. In addition, the pronounced fragility of the rachis of E. canadensis at the time of emasculation made its use as a female parent almost impossible because it was difficult to emasculate at such an early stage without breaking off the weak, pendant rachis. It is quite possible that this species was the female parent of this hybrid which occurs so commonly. In two instances a hybrid plant was found growing immediately adjacent to a plant of E. canadensis with no plants of A. sericeum within 15-20 feet.

Continued effort will be made to produce this cross by isolating plants of E. canadensis surrounded by plants of A. sericeum. If hybridization occurs it can be identified in the progeny. Reciprocal isolations also will be made. Further attempts will be made to make the cross by emasculation and controlled pollination.

Hot water emasculation may prove effective with this material if temperature and duration of exposure are varied. The fact that more selfed seeds were produced when hot water emasculated spikes were pollinated with pollen from the other species cannot be explained at this time. Another unexplainable observation was a more rapid and stronger germination of selfed seeds of E. canadensis resulting after pollination by _A_. _sericeum_, compared with those not pollinated.

Meiosis of _A_. _sericeum_ while predominately regular exhibited lagging chromosomes in 20 per cent and bridges in about 9 per cent of the sporocytes observed. Approximately 10 per cent pollen abortion occurred. Because bridges resulting from chiasmata in heterozygous inversions generally result in the loss of the acentric fragment it is highly probable that a close correlation existed between frequency of bridges and pollen sterility. If such was the case, lagging univalents in most cases must have reached the poles in time to be included in the daughter nucleus. However, that such was not always the case was shown in Figure 15.

Myers and Hill (23), working with autotetroploid grass species found the per cent of quartets showing micronuclei was generally lower than the per cent of nuclei showing laggards at anaphase I and concluded that many laggards were included in the quartet nuclei. Myers (21), working with _Lolium_ _perenne_, found that the majority of anaphase I laggards were included in the daughter nuclei. Thus it is highly probable that most laggards in _A_. _sericeum_ were included in the daughter nuclei and that pollen abortion was correlated with the occurrence of bridges.

On the basis of the limited data available it is not possible to hypothesize the origin of A. sericeum. However, no multivalent associations definitely were observed, and since most polyploid grass species are allopolyploid, it is highly probable that this species is an allotetraploid. Most metaphase I figures were undeterminable and it is possible that multivalent associations occurred but were not observed.

A. sericeum produced a high seed set under isolation. However, it is not known whether under natural conditions the species is self- or cross-pollinated. Considerable variation in gross morphology exists between plants indicating that a certain amount of cross-pollination occurs. Natural crossing to even a limited degree in conjunction with the perennial habit would serve to maintain considerable meiotic irregularity in the species. Natural selection should eliminate such irregularities in a strictly self-pollinated species.

Meiosis in E. canadensis was considerably more regular than in A. sericeum, the only irregularities being the occurrence of bridges at anaphase I. This condition would be expected in a highly self-fertile amphidiploid. Pollen sterility exceeded the frequency of bridges at anaphase I. If the dark staining body found in many diakinesis figures is a supernumerary chromosome, pollen sterility could be partially correlated with that condition. Pollen sterility also could have a genetic basis. However, sufficient data to test these hypotheses are not available.

The material classified as E. canadensis in this study is atypical of the species. However, Lepage (16) and Anderson (3) have classified it as such. Herbarium specimens were submitted to Dr. Jason R. Swallen, Chief Curator, Department of Botany, Smithsonian Institution, Washington, D.C., for identification. The following is a quotation from his reply (40) to the author:

> They are so far from typical E. canadensis, however, that I doubt if they are correctly referred to this species. The spikes are much longer, more lax, and less dense; the awns of the glumes are usually much shorter and, those of the lemmas less flexuous; the spikelets are rather distant, frequently solitary at the nodes, and green, not glaucous. I am not satisfied with calling it E. canadensis, and on the other hand there is no other species in Alaska or North America that it could be . . . . It would seem, then, that this is either a hybrid in itself or a new species, unless it should happen to be an unrecognized Asiatic species. I consider the last rather doubtful. Could it be a hybrid of Agropyron and E. hirsutus?

Stebbins (36) examined the specimens and suggested that "this material may be a fertile derivative from a hybrid between E. canadensis and E. hirsutus."

Anderson (3) reported E. canadensis as an introduction to Alaska, and collected this species in the same area as did the present writer. The most probable means of migration to central Alaska was as an impurity in crop seed or hay. Since the Territory was settled comparatively recently it is probable that the introduction occurred in the past 25-50 years. Further, it most likely would have been introduced from the state of Washington or other western areas where most hay and seed shipped to Alaska originates.

Hitchcock (12) reported both *E. canadensis* and *E. hirsutus* occurring in Washington and other western states. Therefore, the suggestions of Swallen and Stebbins that *E. hirsutus* may be involved in the parentage of the material described herein as *E. canadensis* assume considerable validity. However, until further work is done in determining breeding relationships of this material with typical *E. canadensis* and *E. hirsutus* and an attempt is made to synthesize it from *E. canadensis* and other species, no definite conclusions can be made. Therefore, the designation *E. canadensis* appears justified especially since the material has been so named by Anderson (3) and Lepage (16).

The high degree of meiotic irregularity in *XAgroelymus* is typical of many F_1 interspecific hybrids. Univalents ranged from 0 to 24 per cell and multivalents were common at metaphase I as were bridges at anaphase I. High univalent frequency and occurrence of multivalents were found in the same plant. Although many metaphases were underterminable, the number of multivalents per cell was rather low.

Fourteen per cent of the diplotene and diakinesis figures showed 14 bivalent associations. Many of these were polar views, however, and what appeared to be bivalent associations may have been only loose associations with few if any chiasmata. The fact that only 1 per cent of the metaphase I figures showed 14 bivalents lends support to this assumption.

Although complete pairing was rare, a considerable number of bivalents were observed. Since few multivalent associations occurred in the parent species a considerable portion of this pairing may be considered allosyndetic and therefore the two species may contain genomes that are at least partly homologous.

Multivalent configurations may be the result of translocation of chromosomal segments, especially since some associations appeared to involve more than four chromosomes.

Meiotic irregularities were not sufficient to explain adequately the complete pollen sterility of X<u>Agroelymus</u> since normal figures existed in all meiotic stages. It appears evident that the cryptic structural hybridity hypothesis discussed earlier is the most logical explanation for the complete pollen sterility of this hybrid. Although some chromosomes of the two species are sufficiently homologous to pair they certainly would be expected to differ in small chromosomal segments. Even with a fairly normal meiosis, chiasmata between these partially homologous pairs would produce gametes with unbalanced, disharmonious combinations of chromosomal material. Genetic sterility was eliminated by the partial fertility of the amphidiploids.

Clausen, Keck, and Heisey (8) reported that F_1 hybrids with considerable chromosome pairing produced amphidiploids of low fertility and interspecific segregation. The low fertility of the 19 plants obtained from colchicine treated sectors of the sterile F_1 is in agreement with their statement. However, it should be possible by selection to increase fertility in advanced generations. Progenies from these plants were not available for study with regard to interspecific segregation.

Morphological evidence presented clearly indicates that *A. sericeum* is much closer to X*Agroelymus* than is *A. repens*. With regard to rhizomes, nature of leaf surface, auricles, nature of the surface of the glumes, lemmas and rachillae, the characteristics of *A. sericeum* are reflected in the hybrid while those of *A. repens* are not. The scarious margin of the glumes is wider in X*Agroelymus* than in either *A. repens* or *E. canadensis* and narrower in X*Agroelymus* than in *A. sericeum*. In few if any instances does *A. repens* resemble the hybrid significantly more closely than does *A. sericeum*.

The hybrid exhibits considerable heterosis. Plants are taller, leafier, and they have denser foliage and greater spread than either parent. The number of flowering culms also is greater. In addition, this hybrid begins growth earlier in spring than either parent. These attributes are of considerable value and if sufficient fertility can be obtained in the amphidiploid, X*Agroelymus* may become of economic importance in Alaska.

The use of chromatography as a tool in determining species differences is a rather new approach to the subject. The amount of data reported here and in the literature is insufficient to properly evaluate the technique. More work should be done in this field because it appears to possess considerable potentiality and requires simple equipment, little time and very small amounts of plant material. The results reported here are indicative of the fact that species and various tissues of the same species differ with regard to the amino acid complex. The use of paper electrophoresis also is quite simple and could be used to elucidate differences in protein complexes.

The report of Barrett and McLaughlin (4) that varieties of wheat resistant and susceptible to race 9 of *Puccinia triticina* differed in protein and amino acid content and that resistance and susceptibility might be associated with certain types of proteins is especially interesting. If this proves to be the case, chromatographic and electrophoretic techniques could well become extremely valuable tools in plant breeding programs. It is obvious, however, that much refinement of these techniques and numerous trials correlating results of these techniques with those of conventional methods are necessary. Nevertheless, the advantages of such techniques are so great that they should be more fully explored.

SUMMARY

Certain plants were collected in 1950 at Palmer, Alaska, which on the basis of gross morphology appeared to be hybrids between *Agropyron sericeum* or *A. repens* and *Elymus canadensis*. They proved to be completely sterile and were named X*Agroelymus* by Lepage (16).

Neither hand emasculation and pollination nor hot water emasculation followed by mass pollination was successful in synthesizing the hybrid.

Meiotic studies revealed that *A. sericeum* was quite regular although sufficient irregularities were present to produce about 10 per cent pollen sterility. *E. canadensis* was very regular meiotically but had approximately 25 per cent pollen sterility. Meiosis in X*Agroelymus* was extremely irregular but not sufficiently so to account for complete pollen sterility. The hypothesis of cryptic structural hybridity may explain the complete sterility found in this hybrid. All grasses studied had 28 chromosomes.

Colchicine treatment was effective in producing amphidiploids. Twenty seeds were produced in 1953 and over 40 grams were produced in 1954. Nineteen progenies from treated plants were established in 1954. The per cent of florets that set seed ranged from 0 to 20 in these plants.

Mass collections were used to sample the variation in

A. sericeum, A. repens, E. canadensis, and XAgroelymus. Measurements were reported on a number of morphological characters that could be analyzed quantitatively and descriptive data were reported on others. Data were analyzed with the aid of polygonal graphs and ideographs.

On the basis of morphological evidence, A. sericeum was considered the most likely Agropyron parent of the hybrid. Several characters were cited in which A. repens could not have contributed to XAgroelymus.

Chromatographic analyses were made for content of 13 amino acids in various tissues of the four grasses. In seven instances a particular amino acid was lacking in A. repens and present in the remaining species. This further indicated that A. repens was not a parent of the hybrid. Quantitative values were obtained for nine amino acids but no further conclusions were possible from the data.

The potential value of chromatographic and electrophoretic techniques in this work and in other plant breeding work was discussed briefly.

Considerable vigor, leafiness, and an abundance of flowering culms was found in the hybrid. These valuable attributes may make this hybrid of economic importance but more testing and evaluation are necessary.

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